EXPERIMENTS WITH HIGHLAND *NEPENTHES* SEEDLINGS: A SUMMARY OF MEASURED TOLERANCES

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Introduction

One of the most difficult things to describe to someone is how to grow a plant that is restricted in its tolerances. Partly to blame is our human perception of these conditions. What exactly is "bright light"? What is "clean water" and how acidic is "acid"? Highland *Nepenthes* are often one of the more perplexing groups to work with. What seems to work for one person fails when someone else tries to reproduce the cultural environment. *Nepenthes villosa*, for example, is not an easy plant to grow from seed; it has a narrow range of conditions necessary to grow well. Beans are easy to grow, highland *Nepenthes* are not!

In order to get better insight into the needs of several species, I conducted five years worth of experiments aimed at quantifying their needs. Instead of subjective terms, I used tools to measure environmental conditions in all test groups. A light meter, conductivity meter, pH meter, recording thermometers and a high quality humidity meter were employed to put some numbers behind the findings. (Cheap meters are dangerous to rely on, so only meters that could be calibrated and tested were used.) This way, anyone could nearly duplicate the conditions that were successful, and avoid potential disaster by measuring variables before plants get put into a bad environment.

The following *Nepenthes* were used in this study: *N. burbidgeae*, *N. edwardsiana* (Tambuyukon type), *N. fusca*, *N. stenophylla*, *N. tentaculata* (Mt. Tambuyukon type and Mt. Kinabalu type), and *N. villosa*. All were raised from donated seed or very tiny seedlings (cotyledon spread 0.63 cm., 0.25 inch). Dead or struggling plants were considered indicators of exceeding the species tolerance to one or more variables within their environment. The bane and beauty of using seedlings is that they do not take long to indicate improper environments; they die quickly!

Several points deserve mention, before getting into the "meat" of this article. First, plants grow in a system, where the elements of culture play off each other. For example, higher light levels require greater nutrient levels, as plants need more nitrogen to eliminate photosynthetic (waste) byproducts. Stresses from lower relative humidity might be offset by lower light or more available moisture at the root zone. Thus, I am not suggesting one rigid method, when explaining a successful technique. My intention is to give benchmarks and warning signs.

Second, this article was written as a distillation from five years (1996-2001) worth of records. A full description of these experiments would fill several volumes of this newsletter with tedious detail. My intention is to present a simplified summary of what happened during these experiments. One example of this practical focus was to simplify the complex science of light. The usual grower purchases a readily available light source or uses sunlight. The intensity of the illumination striking the plants is determined by the light source, the distance from the source to the plants, and/or the amount of shading. The simplest way to get a reasonably accurate measurement of intensity is to use a light meter, specifying the unit of measure and the source (very important). For spectral analysis of artificial lights used herein, contact the manufacturer.

Finally, it is important to note that, unlike formal experimental design, there were no control groups, per se. In many of these species, seedling death is the norm. Other growers, who worked with many of the same seed lots, reported complete losses in difficult species like *N. edwardsiana*, and *N. villosa*. Thus, it was nearly impossible to assign a control group without first figuring out how to keep it alive! The main support for these findings is that significantly large numbers of plants were used. The results herein are preliminary and not conclusive; other factors (such as bacterial infection, seed viability, genetics of the donated seed, etc.) could have had an effect on these findings. Readers should consider this article a detailed summary by a professional horticulturist, rather than a classic, formal experiment. As far as I am aware, this is the first time that meters have been used to quantify the major (growing) environmental parameters, and that these measurements have been united in print.

Experimental methods

Seedling lots were from two hundred to four hundred seedlings per species. The seedlings were carefully transplanted into groups of 32 per 10.2 cm (four inch) plastic pot at the cotyledon stage of seedling development. Lesser quantities of seedlings were put into the same size pots when I intended to expose these to extreme, probably fatal conditions (such as pH below 4.5, or light below 4300 lx, i.e. 400 fc), or when the seedlings grew enough to need extra space. These "community pots" were then placed into different, carefully monitored environments (including different media). Groups were examined monthly and compared visually. It was obvious which groups were prospering and which were dead or growing poorly, so I did not measure each plant.

Meters included:

Conductivity meter (American Marine, Inc., Pinpoint™ Conductivity Meter) giving values in microsiemens/cm.

Light meter (Extech, model 40125), values were originally measured in foot candles and converted to lux[1].

Hygrometer/Thermometer (Extech model 44470), values given as relative humidity (RH).

Min/Max thermometers (Taylor), temperatures were originally recorded in degrees Fahrenheit.

Artificial light sources included:

High pressure sodium (Philips, SonAgro, 430W).

Metal halide (Philips, AgroSun™, MS400/HOR/AS, 400W).

A fluorescent combination using 50% Wide spectrum Gro-Lux® (Sylvania, 40W) plus 50% Interior Designer® cool white (Sylvania, 40W).

Greenhouse shading for sunlight was accomplished using Continental Products® Koolray Easyoff. This horticultural liquid shading was diluted/applied/removed as necessary to maintain fairly consistent light levels. Artificial light sources were on for fourteen hours per day (24 hour cycle). Unlike day length supplied by artificial lighting, seasonal day length determined the photoperiod in groups getting sunlight.

All plant groups used in this experiment were given identical preventative fungicide treatment to discourage pathogens from degrading the test groups. A mixture of Zyban® (4 ml/liter, 1 TBS/gal.) plus Subdue® (1.6 drops/liter, six drops/gal.) were lightly misted over seed or seedlings, once every two months. Every other application, Cleary's 3336® (4 ml/liter, 1 TBS/gal.) was substituted for the Zyban to reduce the total chemical load. (Zyban® is the same as Cleary's 3336® plus Mancozeb®.) *Nepenthes* seedlings are subject to damp-off. Usually, damp-off is caused by species of *Pythium* or *Rhizoctonia*. *Botrytis* and several other fungi are usually less common as damp-off culprits. Pathogen identity was primarily determined by examining conidia under compound microscope. Non-systemic fungicides, such as Captan®, were not used because they are less effective and the concentration needed for pathogen control raised the conductivity of the soil to deadly levels[2]. Mortality rate of untreated seedlings (two months after germination) was between ten and eighty percent, so untreated groups were not used in these experiments. Mortality rate of fungicide-treated seedlings was generally below ten percent (two months after germination). Twenty-four hours after fungicides were applied, all media were leached with purified, aerated water, to remove soluble residues.

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[1]Values given in lux can be converted to foot candles using 1 lx = 0.0929 fc.

[2]My thanks to Dr. Jay Stipes, VA Polytechnic Institute, for assisting me in finding an effective fungicide combination.

Another hidden seedling killer proved to be salts that slowly leached out of perlite, one of the main media components. Perlite is hydrated obsidian that is heat treated to expand. Perlite was added to purified water (two parts water to one part perlite) and the conductivity of the water increased more than threefold, over a two day period. Since all examined groups of seedling *Nepenthes* seemed to favor a conductivity level in the soil of between 10-20 microsiemens; the perlite was pushing the conductive load of the media to dangerous levels (24-75 microsiemens). However, after repeated leaching the perlite became a great, inert material to use in seedling media mixes. Certainly, the quality of perlite is variable.

The irrigation water was purified by reverse osmosis and aerated using a utility pump. The conductivity of the water was 10-12 microsiemens. Eighty percent of all groups were watered twice a month with a boiled, strained, peat moss and sphagnum water extract (tea) which was added to irrigation water prior to aeration. The formula, pH and conductivity levels of this diluted tea were monitored and standardized for the tests. The tea was used to add nutrients, regulate soil pH and examine the effects of conductivity in an organic, acid matrix (media containing primarily tannic, fulvic and humic acids as acidifying agents). Conductivity values given here are from water extracts of potting media. Water extracts from the media were made by saturating the media for one hour and passing purified water (approximately equal to the soil volume) through the media. This leachate was measured for pH and conductivity. As one might expect, the makeup of water ultimately determined the conductivity of the media. The breakdown and leaching of organic materials in the media did not elevate conductivity significantly, i.e. organic compounds tend to yield low conductivity products as they leach or decompose. It may be helpful for readers to imagine a hydroponic model, where the water chemistry has more effect on soil chemistry than the media. This is especially true in media consisting largely of perlite. It is also important to note that the effects of conductivity relate to pH in terms of seedling tolerance; if the pH of the media is outside a plant's preferred range, higher conductivity values tend to make the stress worse (higher death rates). When "optimal" conductivity values are given, these are what appeared optimal at suitable pH.

All right, before this sounds too much like a physical science treatise...on to the plants!

Results on specific plants

N. burbidgeae was fairly broad in its tolerances, especially in regards to soil types and temperature. This species tolerated every media blend used. Stunted growth was evident with 50% silica gel, 10% peat moss chunks, and 20% each sphagnum and fir bark. The best growth rates were in media consisting of 50% perlite (leached), 10% peat moss chunks, 10% fir bark and 30% sphagnum moss (dead, long fiber). Media with no fir bark and higher percentages of sphagnum worked equally well. Plants tolerated temperature in the range of 9-41°C (48°-105°F), but grew about 50% slower, with fewer pitchers, when night temperatures were consistently over 18°C (65°F). The best growth occurred when temperatures were 20-29°C (68°-85°F) during the day, and 12-16°C (54°-60°F) at night. Optimal pH was 4.8-5.5. (Slower growth was observed at a pH of 3.5.) Optimal conductivity was 10-24 microsiemens; foliar burn appeared after prolonged exposure (one week or more) to levels above 60 microsiemens. Relative humidity seemed best within the range of 68-95%. Constant high humidity (over 90%) encouraged disease outbreaks and higher death rates, especially in seedlings less than one year old. Preferred light levels were 7000-9700 lx (650-900 fc) under high pressure sodium, 6500-9100 lx (600-850 fc) under metal halide, 5400-7300 lx (500-680 fc) under the fluorescent combination, and 70-93 lx (750-1000 fc) under sunlight. Plants survived lower light levels, but poor pigmentation and etiolated growth was observed. Plants responded well to dilute Miracid® fertilizer , applied monthly to the pitchers[3]. Light misting (foliar feed) of the same solution, monthly, had no noticeable effect on the plants.

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[3]Miracid®, when used, was usually applied at 1/4 the labeled strength for house plants, 0.3 ml/liter approximately equals 0.2g/liter (1/4 tsp/gal. approximately equals 0.75g/gal.). Purified water was used. The solution was put in each opened pitcher, filling the pitchers to the top (peristome).

N. edwardsiana was, without question, the most fragile seedling observed, although the plants became much sturdier as they aged. The following conditions were concurrent with 100% mortality in cotyledon stage seedlings: constant high humidity (over 90% RH), water droplets sitting on leaves or crown, conductivity over 45 microsiemens, and pH above 6. This test group was the smallest (after two months), but a few plants were growing gloriously well in 50% perlite, 10% peat moss chunks, 10% fir bark and 30% sphagnum. In addition, a top dressing of live sphagnum proved very helpful; many of the roots weaved through this layer. Optimal soil conditions appeared to include a pH between 4.8-5.4, and conductivity at levels below 24 microsiemens. Humidity appeared best between 65-85 RH, although older plants (one year or greater) tolerated brief (1-3 days) exposures to 90-99% RH. Under high pressure sodium, light at 7500-9100 lx (700-850 fc) seemed optimal. Other light sources were not tested. Due to the fragile/valuable nature of the few plants, only insects were fed to the plants. When the pitchers were about 3 mm (1/8 in.), dried fruit fly (*Drosophila melanogaster*) larvae were used as food. As the pitchers grew, ants (*Acanthomyops* sp.) were used as food. Plants grew most vigorously when night temperatures were 13-16°C (55-60°F) and day temperatures ran 21-29°C (70-85°F). Growth was very slow for the first 8 months; as the plants reached 2 cm (3/4 inch) in diameter, the growth rate went up dramatically.

N. fusca proved to be very tolerant of cultural variances. None of the test groups showed greater than 25% death rates, except seedlings raised without fungicides, which had from 10% to 100% death rate. This death rate appeared to be random. Although the seedlings survived all the cultivation conditions I subjected them to, some patterns indicating preferences did occur. Media containing higher organic components performed better, i.e. 30% perlite, 10% peat moss chunks and 60% any combination of sphagnum and/or fir bark. A slightly more acidic condition was preferred, from 4.5-5.0 pH. Conductivity tolerances showed good growth between 10-45 microsiemens. Humidity was optimal from 65-90% RH. Though the seedlings tolerated from 10-38°C (50-100°F), growth and pitcher production was poor when nights were routinely over 21°C (70°F). Lighting experiments yielded the following: metal halide, high pressure sodium and sunlight all performed best from 6400-8600 lx (600-800 fc). However, the most vibrant colors were obviously in plants grown under 50% Gro-Lux® and 50% cool white fluorescent at 5400-7500 lx (500-700 fc). Some plants grown for several months under sun, metal halide or hp sodium were moved to the above fluorescent combination with dramatic results; green leaves turned bronzy, light reds became vibrant magenta and spotting on the pitchers became much darker. Growth rates, under all light sources, were similar. Miracid® at 1/4 strength (see Footnote 3), applied inside pitchers, was effective. Ants worked as well for feeding.

N. stenophylla was the slowest overall performer, and I am not certain I ever found ideal conditions for growing it. Generally, the plants grew reasonably well but very slowly, although about 10% of the plants, within a group, tended to grow faster than the others. Despite the sluggish growth, the plants appeared healthy. Pitcher feeding seemed very important, but not all plants responded similarly; about 30% of a group grew 200% in six months, whereas the rest of the seedlings grew about 20% in six months. Miracid® (see Footnote 3), *Drosophila* larvae and ants were equally effective in feeding tests. Despite somewhat ambiguous results, plants grew reasonably well in media containing 40-50% perlite and 40-50% sphagnum moss; adding about 10% fir bark had no noticeable effect. Conductivity was best near 10-22 microsiemens; stress appeared at levels over 45 microsiemens. The pH observations were inconclusive. One plant (in a container with 5 other similar seedlings) took off at pH 4.0 , while the rest appeared impaired. Most plants grew reasonably well at a pH of 5.0. All light sources worked well, but the best observed growth was under sunlight at 8600-12,000 lx (800-1,100 fc). Plants seem to resent water on the foliage and crown, when young; nearly 30% died if their tops were wetted routinely. Plants grew well at 65-90% RH and tolerated temperatures of 10-38°C (50-100°F). Best growth was achieved with nights at 13-17°C (55-62°F) and day temperatures of 24-29°C (75-85°F). Worth noting, the gradual appearance of mosses on the media surface appears to coincide with increased growth rate/plant health.

N. tentaculata from Tambuyukon and from Mt. Kinabalu behave like two different species. Though I am trusting the accuracy of the donors, the morphology of the plants supports the original, supposed ranges. Both types showed an affinity for acidic media, pH 3.8-5.0. In several cases, plants growing near a pH of 4.0 did better than those at higher pH. This was observed in seedlings of Tambuyukon origin; Kinabalu type plants performed fairly equally from pH 3.8-5.0. Peat tea was found more useful in maintaining a lower pH and overall health in Tambuyukon plants. Both groups tolerated conductivity ranges from 10-40 microsiemens. A media of 30% perlite with 20% peat moss chunks and 50% sphagnum worked well for both types. Since these plants showed affinity to low pH, a few of each type were potted in pure peat moss, which had the dustier fraction removed. Despite early success, all plants grown in pure peat were dead after one year, whereas plants in better aerated media survived. As temperature ranges were increased, the differences in Tambuyukon and Kinabalu plants became dramatic. Kinabalu plants were much more tolerant of higher temperatures (10-37°C, 50-98°F). The Tambuyukon plants started dying when either the day temperature went much beyond 29°C (85°F) or the night temperatures stayed above 18°C (65°F). Whereas other species usually showed stress signs before dying, *N. tentaculata* from Tambuyukon often died overnight, with little or no warning. I could not help but be reminded of how *Darlingtonia* suddenly collapses after persistent warm media. Evident, as well, was a relationship between light and temperatures: the higher the temperature, the lower the light tolerance in *N. tentaculata* from Tambuyukon. Both races grew well under 6500 lx (600 fc) of the previously mentioned fluoreseent light mix. Under high pressure sodium, 5400 lx (500 fc) was effective. Tambuyukon plants did poorly, but survived under metal halide (no optimal range observed), whereas Kinabalu types thrived at 6500-7500 lx (600-700 fc) of metal halide. Both types did well receiving from 6500-9100 lx (600-850 fc) of diffused natural light. Under the above light levels, Kinabalu plants thrived from 14-32°C (58-90°F); Tambuyukon plants did best from 10-26°C (50-78°F). In this study, *N. tentaculata* from Tambuyukon was the most sensitive species to high temperature, even more sensitive than *N. villosa*. It may be that the dark green leaves are subject to high heat gain, or that symbiotic relationships, especially at the root zone, are greatly influenced by temperature. Chelated iron availability may also be critical, as distressed plants showed foliar symptoms similar to iron deficiency. More study on this species is needed.

N. villosa had some unusual preferences in culture. For a cloud forest dweller, this species showed a high death rate when seedlings were misted or kept at very high humidity (> 90% RH) for long periods (a week or more). Optimum humidity levels were 65-85% RH. Though the plants tolerated warm nights to 24°C (75°F), the plants stopped growing well when nights were above 17°C (62°F). Many seedlings stayed the same size for over one year, when constantly exposed to nights between 17-24°C (62-75°F); most of the plants in this group ultimately died. The best plants were grown with night temperatures near 14°C (58°F) and day temperatures from 21-29°C (70-85°F). *N. villosa* proved very sensitive to conductive water or media, needing a range below 25 microsiemens to grow well. Seedlings exposed to foliar Miracid® at 1/4 strength and 1/6 strength died within two weeks (see Footnote 3). Seedlings 3/4 inch or more in diameter, however, responded well to the above solutions when placed only in half (some) of the pitchers. Ants proved to be effective, as did dried *Drosophila* larvae, Because of the low nutrient level of the media, pitcher feeding appeared critical for plant health. Since the tiny seedlings make pitchers too small to feed with insects (practically speaking), I developed a technique to deliver the dilute Miracid® into pitchers 3mm (1/8 inch) tall, or less. Hair, from Whitetail deer (*Odocoileus virginianus*), was clipped to 3mm (1/8 inch) and soaked in the fertilizer. Since deer hair is porous in all directions, it proved an effective carrier for the nutrients. One or two hairs could easily be put into a tiny pitcher, once the pitcher lid was removed. Of the seedlings given this treatment, 75% showed significant growth rate increase. However, the other 25% usually died. When media with significantly higher organic/nutrient levels were used, the plants died. Often the low nutrient uptake of the seedlings created an ideal environment for blue-green algae to form a pellicle on the media surface. Aside from possible phytotoxic compounds, this pellicle decreased oxygen supply to the roots. In every test lot, *N. villosa* displayed an affinity for extremely well oxygenated media, or media with very high porosity. The best plants were raised in 75-80% perlite, with the remaining fraction sphagnum and peat moss chunks. Peat tea was used periodically to maintain a pH of 4.8-5.0 and a conductivity of 10-18 microsiemens. Plants given media at pH 3.8-4.0, (conductivity 28-41 microsiemens) were stunted. A thin top dressing of live sphagnum seemed to help, but was not as useful as in *N. edwardsiana* culture; the *N. villosa* did not root (much) directly into the live moss.

Figure 1: Tiny *Nepenthes villosa* seedlings being manipulated with a pair of forceps.

Although other growers have been successful using metal halide as artificial lighting, I was not able to get satisfactory growth with ranges of 8100-12000 lx (750-1100 fc). Much better growth was achieved using natural sunlight 8600-11000 lx (800-1000 fc), the fluorescent light combination mentioned earlier 7300-8100 lx (680-750 fc), and high pressure sodium 8400-9700 lx (780-900 fc). When a single fluorescent (2-40 watt bulbs, as described earlier) was angled to add to the high pressure sodium spectrum, colors and general plant health was improved. This is interesting, since the fluorescent lights only added about 900 lx (80 fc), when used in this fashion. Plants growing under the high pressure sodium/fluorescent combination grew faster and produced more pitchers.

Concluding comments

Shortly after this study was completed, Dr. Perry Malouf (personal communication[4]) did some limited soil analysis on Mt. Kinabalu, examining samples from near the root zones of several *Nepenthes* species. His preliminary findings on conductivity and pH in the field were very similar to those found to be near optimal in these seedling experiments. In general, *Nepenthes* seem to favor a soil pH near 5 with very low conductivity.

It is hoped that this information will help growers achieve success in their efforts and that ecologists can use the quantified data such as acid tolerances and conductivity to better predict when a *Nepenthes* habitat is threatened by factors such as acid rain, fertilizer runoff or deforestation. Acid rain can have a pH less than 3, and I have measured conductivities of over 70 microsiemens in Virginia's polluted rainwater. I am concerned that the increased use of high sulfur coal, as fuel in China and surrounding countries, will introduce acid rain to highland habitats. The reckless disregard for pollution, that has killed fish and most of the high elevation spruce forests in Virginia, could manifest itself in places like Mt. Kinabalu and Mt. Tambuyukon, transforming the currently vibrant ecosystems into ecologically damaged mountainsides. With the rapid development of the Eastern hemisphere, I fear many of us will live to witness such a tragedy. Monitoring rain water quality in montane habitats may prove vital in preventing habitat destruction by an invisible threat. Minimizing the risks is key to avoiding loss of species diversity.

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